In silico screening of potential inhibitors against Glutathione S Transferase of Plasmodium Falciparum.

Thesis submitted in partial fulfilment of the requirements for the degree of

Bachelor of Technology

In

Biotechnology

By

Vishnu Murthy Appala

(110BT0592)

Under the guidance of

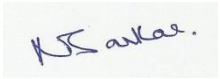
Dr .Nandini Sarkar

Department of Biotechnology and medical Engineering
National Institute of Technology
Rourkela-769008, Odisha, India

Dr. Nandini Sarkar
Assistant professor
Department of biotechnology and medical engineering
National institute of technology, Odisha, India

Certificate

This is to certify that the thesis entitled "***In silico* screening of potential inhibitors against Glutathione S Transferase of Plasmodium Falciparum**" by **Vishnu Murthy Appala (110BT0592)** submitted to the National Institute of Technology, Rourkela, for the degree of Bachelor of Technology is a record of bona fide research work, carried out by her in the department of Biotechnology and Medical Engineering under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university/ institute for the award of any degree or diploma.



Dr. Nandini Sarkar

Assistant Professor

Department of Biotechnology and Medical Engineering, NIT Rourkela, 2014

ACKNOWLEDGEMENT

If words are reflected as symbols of appreciation and token of acknowledgement, then words play the role of thanks to exhibit the deeply embedded feeling of gratitude. I owe special dept. of gratitude to my guide **Dr. Nandini Sarkar**, Department of Biotechnology and Medical Engineering for her constant support and guidance throughout the course of the work. Further I would like to express my thankfulness to **Arun Kumar Maurya**, for his constant support and advice in my project work.

It is an immense pleasure to thank my friends **N Sai Venkata Sarath Chandra, Siddhant Mohanty, Mahesh Nagwan, Neha Goyal** and all others for their persistent encouragement and day –to-day support.

I would also like to thank **Mr. Shubankar Paul** for his support and guidance.

I sincerely thank all my faculty members for their blessings.

Finally I am grateful to my parents **Mrs. Rajyalakshmi**, **Mr Appala Subba Rao** and family members for their endurance, love, wishes and support, which helped me in completion of my work. Above all, I thank **Almighty** who showered his blessings upon us.

(Vishnu Murthy Appala)

CONTENTS

LIST OF FIGURES AND TABLES

ABSTRACT

Glutathione S Transferase catalyse the conjugation of glutathione with a wide mixed bag of hydrophobic mixes, for the most part bringing about nontoxic items that could be readily killed. As opposed to different organisms, the malarial parasite Plasmodium falciparum has one Glutathione S Transferase enzyme (Pfgst). This Glutathione S Transferase is highly found in the parasite, its movement was discovered to be increased in chloroquine cells, and it has been indicated to go about as a ligand in for parasitotoxic hemin. Thus, the enzyme speaks to a guaranteeing target for antimalarial drug development. The target is docked with small ligand molecules with AutoDock Vina. The ligand molecules are checked for their toxicity Pfgst has a shorter C-terminal area bringing about a more dissolvable-available binding site for the hydrophobic substrates. The ligand molecules selected for docking are used for the inhibition of the Glutathione S Transferase molecule. The ligands that are selected are chloroquine, S hexylglutathione, Artemesinin, Hemin, Protoporphyrin IX, Tetracycline, Quinidine and Quinine. The ligand molecules are found to inhibit the Glutathione S Transferase molecule in vivo. These findings thus suggest that above molecules can may be potentially used for the drug development of malaria.

Keywords: Malarial disease, Glutathione S Transferase, enzyme inhibition, drug target.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Malaria is a genuine illness that causes a high fever and chills. You can get it from a bite by a contaminated mosquito. Malaria is uncommon in the United States. It is frequently found in Africa, Southern Asia, Central America, and South America.

Malaria is created by a bite from a mosquito contaminated with parasites. In exceptionally extraordinary cases, individuals can get malaria on the off chance that they come into contact with contaminated blood. A creating embryo may get the illness from its mother. You can't get malaria simply by being close to an individual who has the disease.

Most malaria contaminations cause side effects like flu virus, for example, a high fever, chills, and muscle torment. Side effects have a tendency to travel every which way in cycles. A few types of malaria may cause more genuine issues, for example, damage to the heart, lungs, kidneys, or cerebrum. These types could be destructive.

Glutathione S-transferases (GST) are a family of stage II detoxification enzymes catalysing the conjugation of glutathione (GSH) to an extensive assortment of electrophilic substrates [2]. The enzymes are rich in most living beings examined, going from prokaryotes to well evolved creatures [7]. The less toxic and more hydrophilic results of GST catalyzed responses could be in partially metabolized and discharged, accordingly ensuring cells against cytotoxic and gene toxic mixes [5]. Additionally conjugation responses, a few GST can catalyse GSH-dependent reduction of hydro peroxides produced, e.g., throughout oxidative stress. Notwithstanding their enzymatic capacities, GST can bind to an extensive variety of endogenous and exogenous ligands, for example, hormones and bilirubin and in addition drugs and pesticides, which regularly impair the catalytic action of the enzyme [3]. In the course of the most recent years GST research has concentrated on resistance phenomena in insect strains and multidrug-resistant tumour cells. Because of their enzyme-specific overexpression in different tumours GST have developed as promising therapeutic targets, and a basis to use GST inhibitors in mix with alkylating executors to go around resistance has been built. A case for GST being included in resistance to insecticides are the DDT-metabolizing enzymes of the malaria vector Anopheles [1].

Tropical malaria, which is created by the protozoan parasite Plasmodium falciparum, is answerable for ~515 million clinical cases and one to three million deaths every year. The development and spread of drug resistance to normally utilized chemotherapeutics are main considerations contributing this expanding load [8].

GST activity has been located in all Plasmodium species considered so far and additionally in all intra erythrocytic phases of the parasite [5]. Pfgst has been evaluated to speak to >1% of the aggregate cell protein. A part of GST from P. falciparum (Pfgst) in the advancement of drug resistance in malarial parasites has been proposed and is controversially examined [10]. The essential structure and additionally the three-dimensional X-beam structure of Pfgst vary essentially from human Gsts, and demonstrate that Pfgst can't be assigned to any of the previously known GST classes, subsequently speaking to a novel GST isoform. Since, besides, the parasite harbour one and only GST and inhibition of Pfgst is relied upon to aggravate GSH-subordinate conjugation methods, to enhance levels of cytotoxic peroxides and to increase concentration of toxic ferriprotoporphyrin IX (FP), Pfgst is a most promising drug target [9].

Identification of the area of ligand binding sites on a protein is of major importance for a reach of provisions including molecular docking, drug designing and structural identification and likewise helps in correlation of functional sites [6]. Now-a-days the improvement of bioinformatics is preparing for simple investigation of protein-ligand interaction. Computational biology and bioinformatics can possibly accelerate this methodology and likewise it helps in reducing the expense [4]. Docking is method which predicts the preferred orientation of one molecule to an alternate molecule when they are bound together to structure a stable complex molecule. Here two molecule bind to one another in a three dimensional space. Docking might be referred to as "lock and key" model. Here the protein can be a lock and the ligand might be called as key. There are different tools utilized for docking studies with the assistance of bioinformatics. Utilizing the bioinformatics tools time and expense of living work reduces definitely.

CHAPTER 2

LITERATURE REVIEW

2.1 STRUCTURE OF GLUTATHIONE S TRANSFERASE

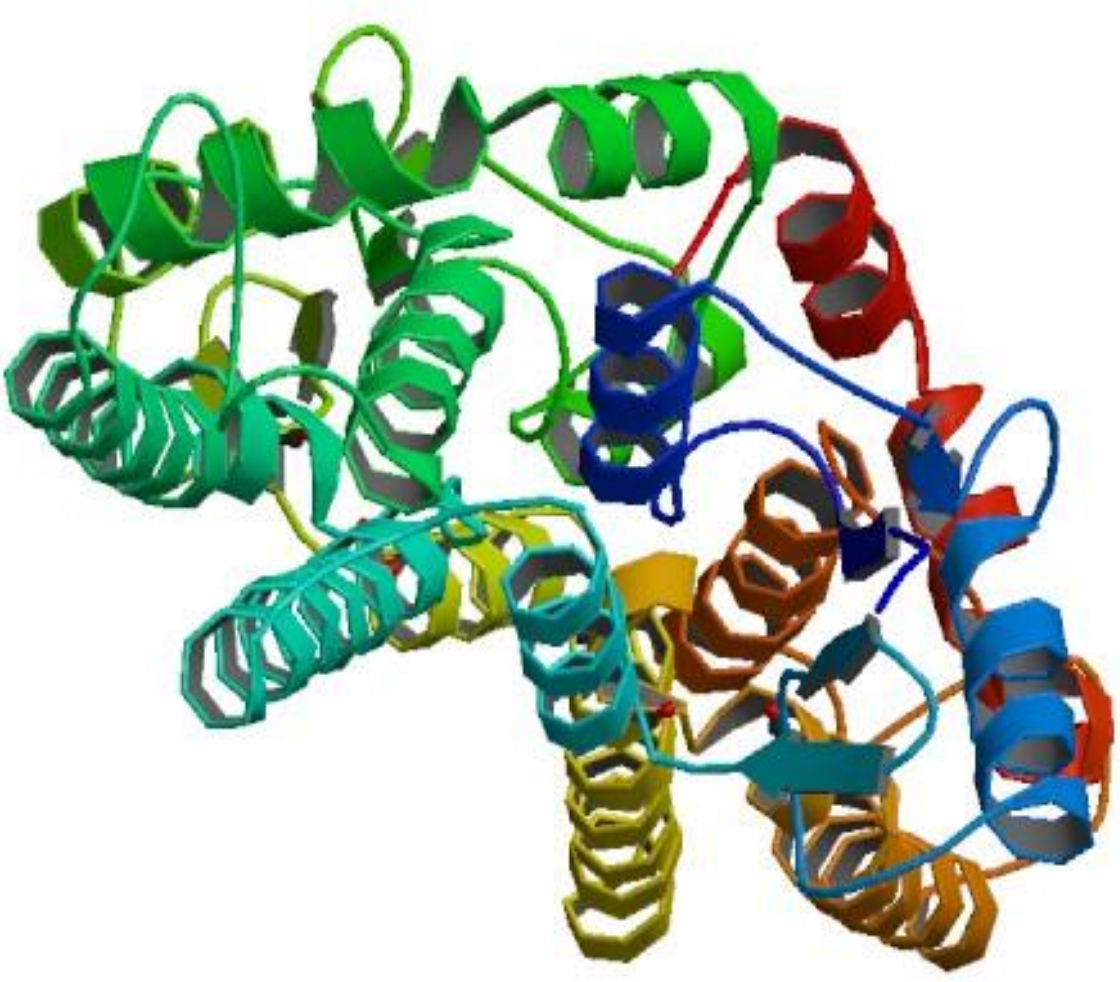


Figure 1. Asymmetric structure of Glutathione S Transferase(1OKT)

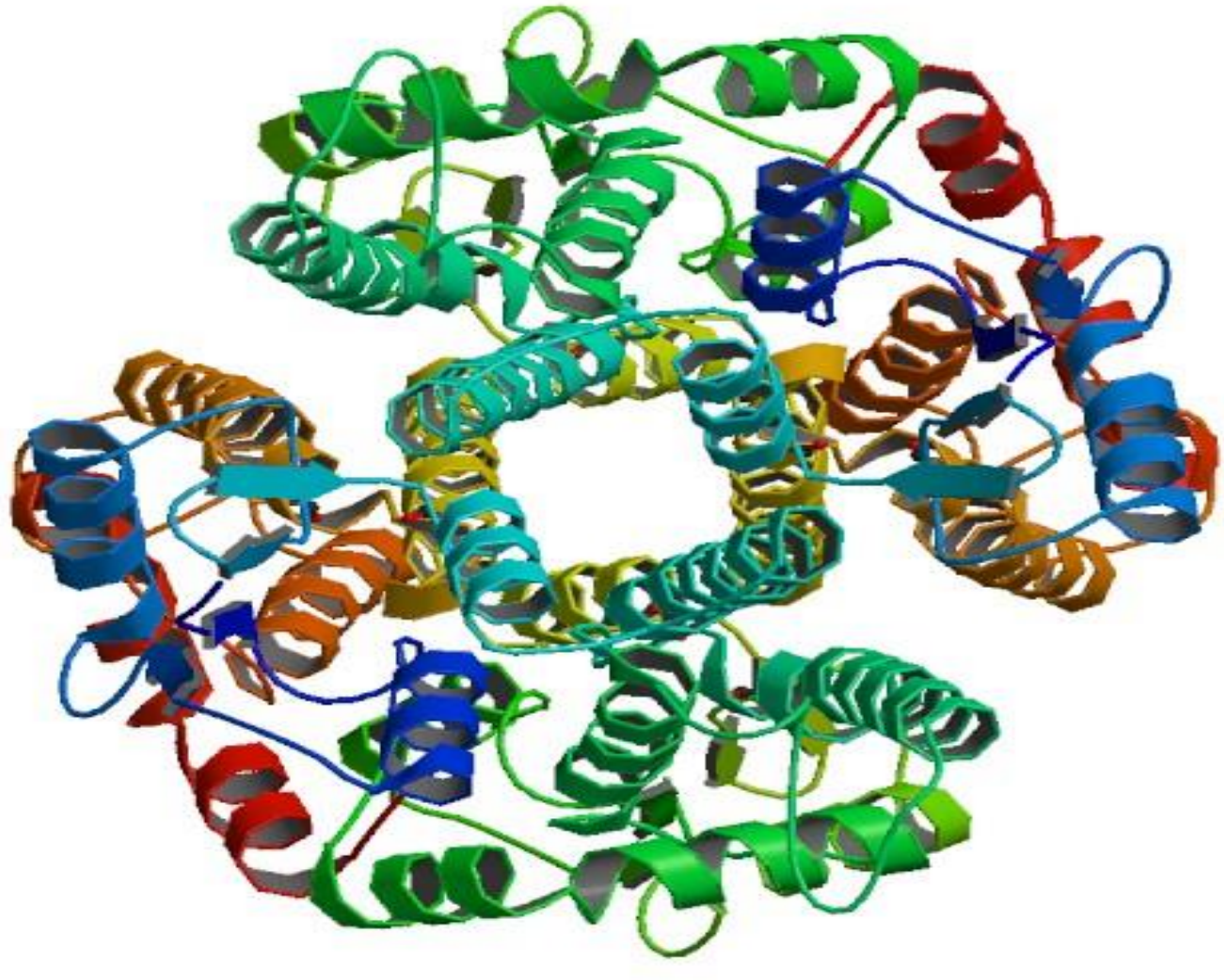


Figure 2. Assumed Biological molecule of Glutathione S Transferase(1OKT).

2.2 NOVEL DRUG DISCOVERY STRATEGY

Drug discovery and advancement is exceedingly complex and, extensive and process. For another ligand to arrive at in the market as potential inhibitor must create by the methodology which is normally known as developmental chain or "pipeline: and comprises of various different stages. For the most part it might be assembled under two stages, early pharmaceutical exploration and late pharmaceutical R&D. Early pharmaceutical examination includes two steps prepare in which distinguishing proof and displaying of the biological target inside the body (the protein) is the first step, emulated by the second venture of ID of lead compound (ligand) that shows drug like properties concerning this protein. Later, the drug

experiences numerous periods of clinical improvement in people. In the clinical stage, the drug is directed first to creatures and after that to human volunteers to focus:

- The channel of the drug through the body- beginning the time when it is controlled in body to the time when it is discharged from the body.
- Drug's impacts on the body.
- Its proficiency to cure the sickness being dealt with.
- Adverse reactions of the medication.

In silico drug design or CADD (Computer Aided Drug Design) system fills this examination prerequisite. Moreover, price water house coopers states that "the general expense of drug development could be decreased by to the extent that half through broad utilization of in silico innovations in drug discovery procedure". Also, studies from quantum mechanics, sub-atomic elements and sub-atomic dockings.

In silico drug finding methodology embody three stages:

(i) First stage includes distinguishing proof of a helpful target and building a heterogeneous Little particle library to be tried against it, emulated by the improvement of a virtual screening convention initialised by either docking of small particle from the library or building these structures in the active site by De novo design technique.

(ii) In second stage, these selected hits are checked for specificity by docking at binding destinations of other known drug targets.

(iii) In third stage, these selected hits are subjected to detail in silico ADMET profiling studies and those molecules that pass these studies are termed as leads.

2.3 DOCKING OF PROTEIN AND DRUG MOLECULES BY AUTODOCK TOOL

Auto Dock is widely used in the prediction of bimolecular complexes for structure & functional analysis and in molecular design. It combines an empirical free energy force field

with a Lamarckian Genetic Algorithm (LGA), providing fast prediction of bound conformations with predicted free energies of association.

Auto Dock was released in 1990 and consistently from early time it has proven to be an effective tool. It can predict accurately and quickly the bound conformation and binding energies of ligand with macromolecular targets. The primary algorithm used by Auto Dock for conformational searching is the Lamarckian Genetic Algorithm (LGA). The "Lamarckian" allows individual conformations to search their local conformational space, to find local minima, and ultimately pass this information to later generations. AutoDock4 is also incorporated with simulated annealing (SA) search method and a traditional genetic algorithm (GA) search method. It uses a semi empirical free energy force field to predict binding free energies of small molecules to macromolecular targets and shows a standard error of about 2-3 kcal/mol in prediction of binding free energy in cross-validation studies.

2.5 STUDY ON AVAILABLE SMALL LIGAND MOLECULES.

ARTEMESININ

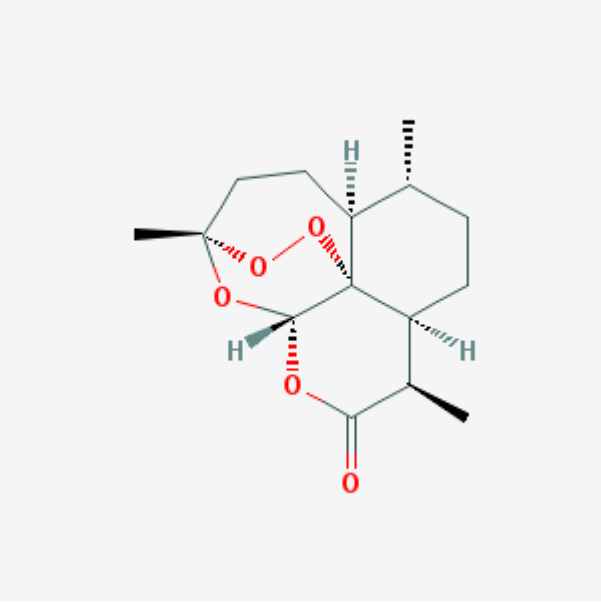


Figure 3.Structure of Artemesinin from PubChem database.

Artemesinin and its similar compounds are used widely all over the world because these compounds appear to be involve in heme-mediated decomposition of peroxide bridge to produce free radicals that are carbon centred. The involvement of heme explains the toxicity in the matter of malarial parasites. The resulting free radicals are alkylate hem end proteins, of

which there are translationaly controlled tumour proteins. Clinically relevant artemesinin has not yet been demonstrated.

CHLOROQUINE

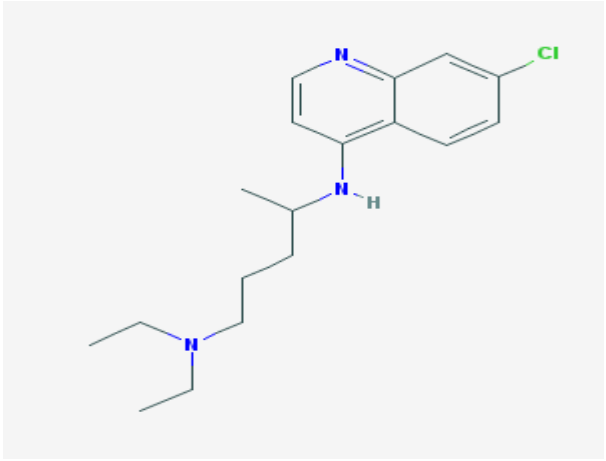


Figure 4. Structure of chloroquine from PubChem database.

The uptake of chloroquine by Plasmodium Falciparum parasites has been attributed to specific carrier-mediated transport. Chloroquine is transported in the place of protons by the membrane of parasite Na+/H+ exchanger where it inhibits the polymerization of hemin, which allows the accumulation of toxic haemoglobin and kills the cell.

HEMIN

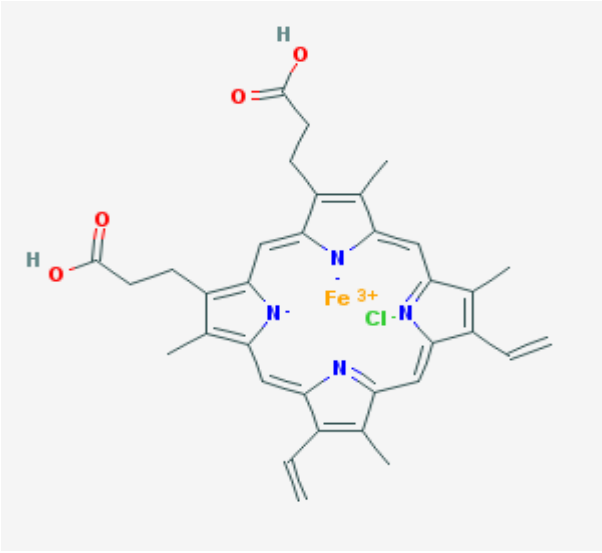


Figure 5. Structure of hemin from PubChem database.

Malarial parasites that are isolated from the mouse erythrocytes are further lysed by hemin or by chloroquine-hemin complex amounts that could be produced by release of less than 0.1 percent of the heme in the haemoglobin of the erythrocytes. This effect of hemin may explain the protection against malaria that is provided by thalassemia and various other conditions causing denaturation intracellular in haemoglobin.

PROTOPORPHYRIN IX

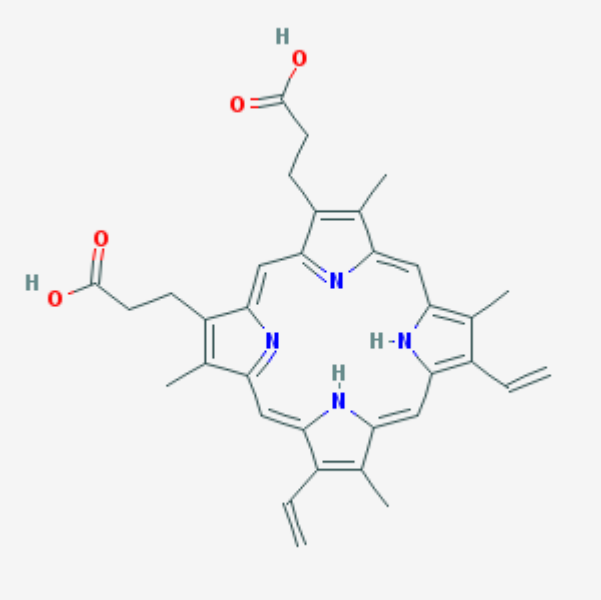


Figure 6. Structure of Protporphyrin IX from PubChem database

The erythrocytes of Plasmodium Falciparum parasite converts most of the host haemoglobin heme into nontoxic crystal. This work examines a binding mechanism for zinc inhibition of heme crystallization which is similar to the quinolones that are antimalarial. Protoporphyrin IX never forms crystal only and never extends on already formed heme crystals.

PYRIMETHAMINE

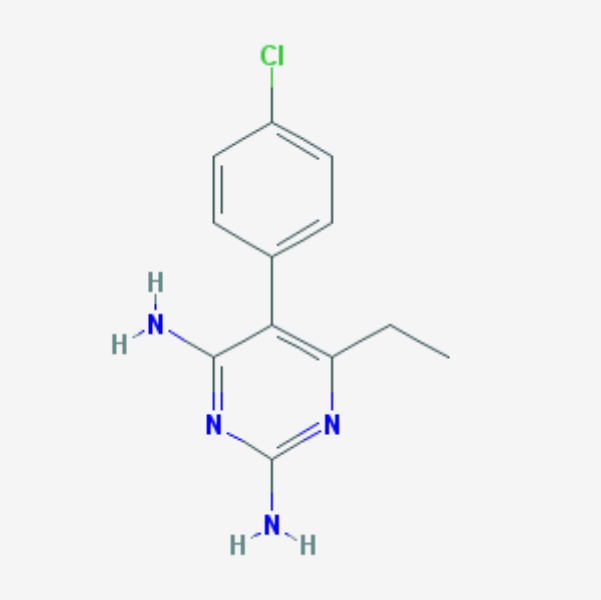


Figure 7. Structure of Pyrimethamine from PubChem database.

The trails that are therapeutic with Pyrimethamine have indicated that the drug could effectively control a clinical attack of vivax or other malarial infection. In two or three days but it might fail in certain percentage of Plasmodium falciparum infection.

QUININE

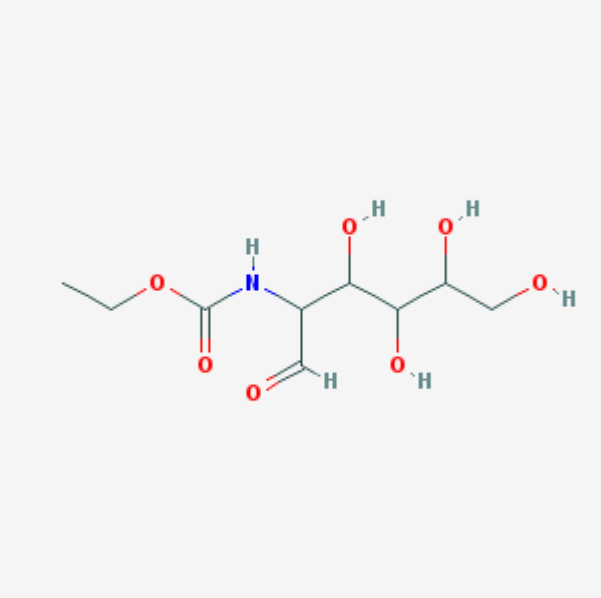


Figure 8. Structure of Quinine from PubChem database.

Quinine and the other derivative drugs are effective treatments for severe falciparum malaria. Trails have not demonstrated convincing evidence of a mortality advantage for quinine. These results can be probably generalised to the treatment of severe malaria in adults from all areas especially in those areas that with the hyperparasitemia.

TETRACYCLINE

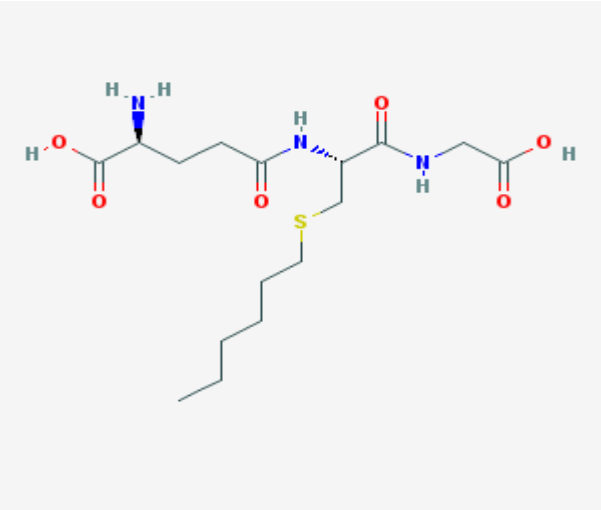


Figure 9. Structure of Tetracycline from PubChem database.

Tetracyclines are very effective but they are slow-acting antimalarial drugs whose mechanism of action remains uncertain. To characterize the mechanism of tetracycline, we evaluated their activities, impacts on parasite transcription, and its effects on two targets, apicoplast and the mitochondria, in cultured Plasmodium falciparum.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 SOFTWARES USED

3.1.1 Pubchem

3.1.2 NCBI

3.1.3 Swissmodel.exapsy.org

3.1.4 PubMed

3.1.5 PDB (rcsb.pdb.org)

3.1.6 Chimera

3.1.7 MGL Tools

3.1.8 Auto Dock Tools

3.1.9 Auto Dock Vina

3.1.10 Swiss PDB Viewer

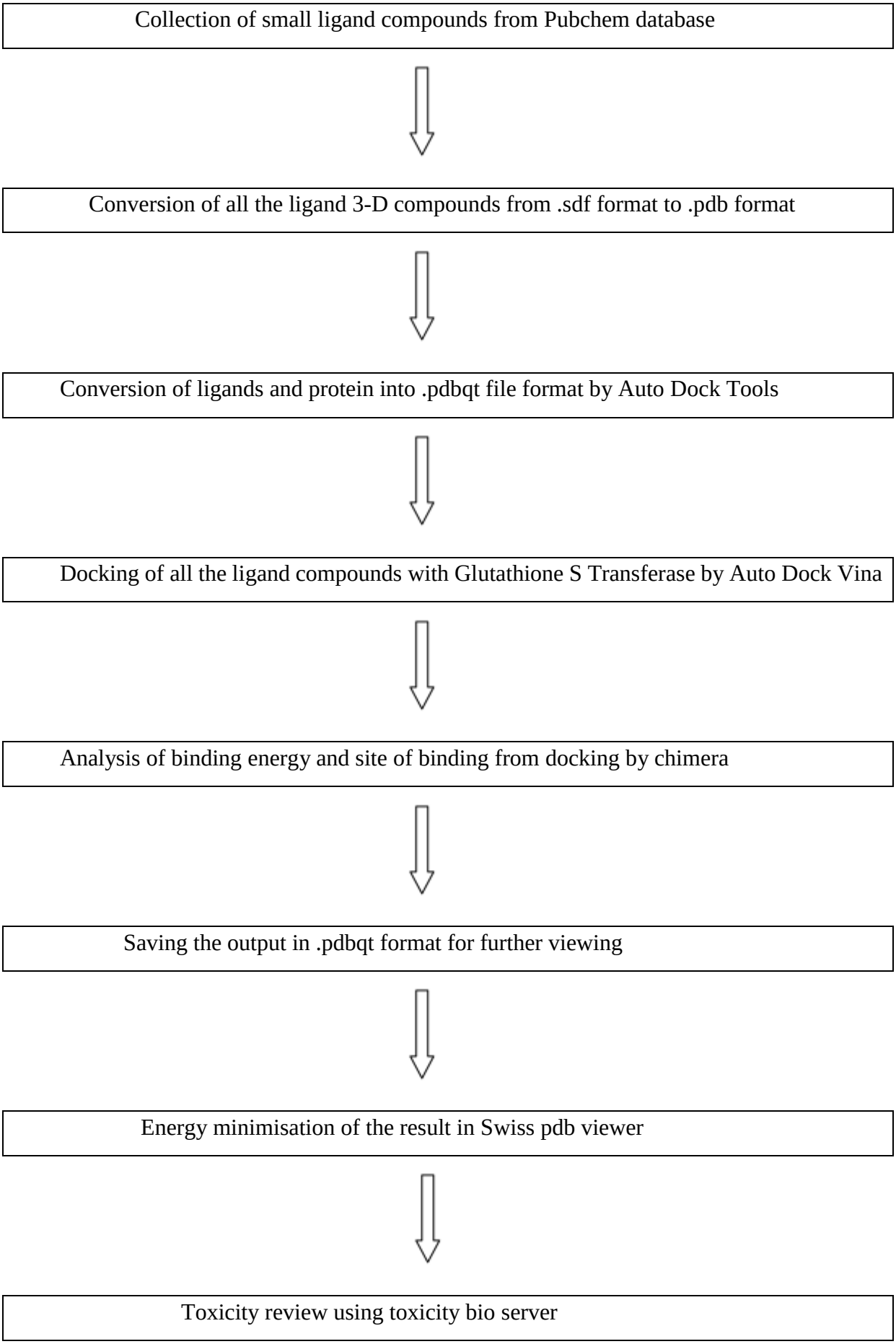
3.1.11 Toxicity Bio server

3.1.12 CastP server

3.2 PROTOCOL FOLLOWED

3.2.1 Inhibition of Glutathione S Transferase

Retrieval of 3D structure of 1OKT protein from PDB


Collection of small ligand compounds from Pubchem database
Conversion of all the ligand 3-D compounds from .sdf format to .pdb format
Conversion of ligands and protein into .pdbqt file format by Auto Dock Tools
Docking of all the ligand compounds with Glutathione S Transferase by Auto Dock Vina
Analysis of binding energy and site of binding from docking by chimera
Saving the output in .pdbqt format for further viewing
Energy minimisation of the result in Swiss pdb viewer
Toxicity review using toxicity bio server

Analysis of 2D structure using ligplot+

3.3 METHODOLOGY

PDB (protein data bank) provides us with the 3-dimentional structural data of macromolecules such as proteins and nucleic acids. This was founded in Brookhaven National Laboratories (BNL). This is of much importance because the understanding of the structure of the compounds is the basic step.

The Glutathione S transferase protein with the PDB ID 1OKT was downloaded

Figure10. Homepage of PDB database where 1OKT was downloaded.

3.3.1 Retrieval of small ligand molecules from PubChem database

PubChem is a database which provides us with structures of small organic molecules. It also contains information related to its origin and related literatures. It is updated by NCBI. Compounds are freely downloaded in .sdf file format or chemical (CID) format.

a) The URL for the site is pubchem.ncbi.nlm.nih.gov.

b) In the search box name of the inhibitors was typed.

c) The 3-D structures was retrieved in .sdf file format.

3.3.2 Conversion of all the ligand 3-D compounds from .sdf format to .pdb format

UCSF Chimera (or simply **Chimera**) is an extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, assemblies, sequence alignments, docking results, trajectories, and conformational ensembles.

a) Click on “File” and then “open” and browse for ligand molecule.

b) Now convert it to .pdb by click “Save as .pdb”.

3.3.3Conversion of ligands and protein into .pdbqt file format by Auto Dock Tools.

Auto Dock is currently maintained by The Scripps Research Institute and Olson Laboratory.

a) Select the protein molecule in Autodock tools by clicking on ”File” and “Open”

b) Click on “Edit” and remove Water molecules.

c) Click on “Edit” and polar hydrogen molecules only.

d) Now click on “File” and “Write PDBQT” and save.

e) Now select small ligand molecule by clicking on “Ligand” and “Open”

f) Click on Ligand and “Choose Root”

g) Click on Ligand and “Detect Root”

h) Now clicking on File and “Write PDBQT” it is saved.

i) All the files are kept in one folder for further use.

3.3.4 Docking of all the ligand compounds with Glutathione S Transferase by Auto Dock Vina

Auto Dock Vina is a software used for the process of docking of the protein molecule with the small ligand molecules. The molecules which are already prepared by using the MGL tools are taken and they are docked using this software. The coordinates of the active site predicted as Tyrosine at the position of 9 of the chain are given. The target is docked at this site with the small ligand molecules using Auto Dock Vina.

3.3.5 Toxicity Prediction by Chem Bio server

This is a Server which is part of the Bio academy **Bio server** that contains some tools and web services developed in the Biomedical Research Foundation of the Academy of Athens. Its main aim is to improve computational molecule screening and analysis and it is financed by the Greek Ministry of Education Cooperation Proposal Entitled as PIK3CA Oncogenic Mutations in Breast and Colon Cancers.

a) The ligand molecule in either .mol or .sdf format is uploaded
b) Then on clicking "Process Data" at the end of the page, it gives the result whether the compound is toxic or nontoxic.
c) If the compound is toxic, it shows because of which molecule.

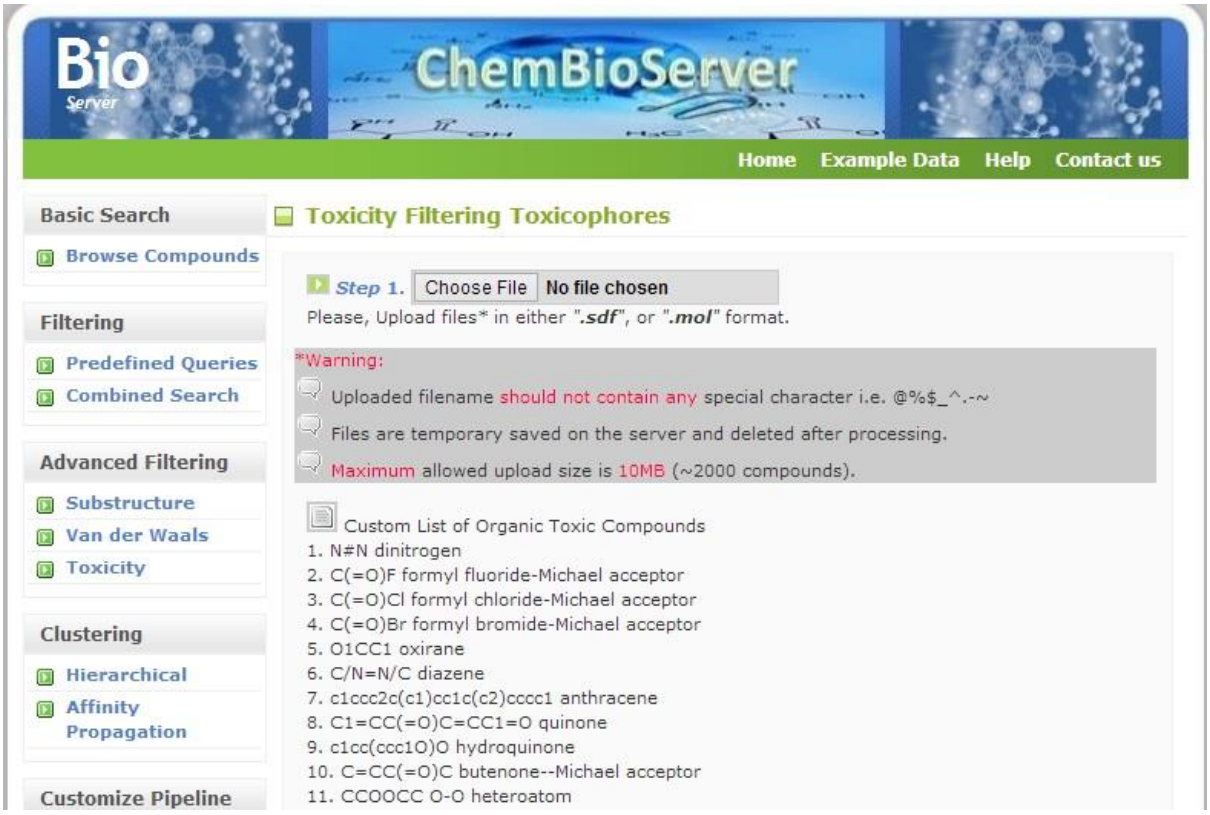


Figure 11. Homepage of Toxicity Bio server online.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 RESULT

When present as a trophosite in human erythrocytes, the malarial parasite Plasmodium Falciparum exhibits an intense glutathione metabolism. Glutathione plays a role not only in anti-oxidative defence and in maintaining the reducing environment of cytosol. Many of the known glutathione dependent processes are directly related to the specific lifestyle of the parasite. Reduced glutathione supports rapid cell growth by providing electrons for de oxyribose nucleotide synthesis and takes part in detoxifying heme, a product of haemoglobin digestion. Cibacron Blue, an inhibitor of the structurally known P. falciparum Glutathione reductase, appears to be a promising antimalarial medication when given in combination with chloroquine.

Inhibitory compounds were selected which were already reported to inhibit the Glutathione S Transferase protein in vivo. The compounds selected were artemesinin, chloroquine, cibacron blue, hemin, protoporphyrin IX, pyrimethamine, quinidine, quinine, S hexylglutathione and tetracycline.

To identify the binding sites of glutathione s transferase, a server castP was used and the binding site of Tyrosine (TYR) at the position 9 of the chain B is selected.

The docking is done with the Autodock Vina tool and results were observed.

The result of docking with ligand molecule Artemesinin

Table 1

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-12.0	0.000	0.000
2	-11.9	1.770	3.397
3	-11.5	1.461	3.582

4	-11.5	1.673	2.889
5	-11.4	1.600	2.543
6	-11.2	1.525	3.241
7	-11.1	11.599	12.585
8	-11.1	11.532	12.457
9	-11.1	1.044	2.781

The result of docking with ligand molecule chloroquine.

Table 2

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-8.6	0.000	0.000
2	-8.4	1.213	2.098
3	-8.2	1.802	2.255
4	-7.7	12.998	13.938
5	-7.7	14.043	15.826
6	-7.7	1.681	3.093
7	-7.5	15.583	17.355
8	-7.5	14.973	16.645
9	-7.5	3.009	6.947

The result of docking with the ligand molecule cibacron blue.

Table 3

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-12.5	0.000	0.000
2	-12.4	22.408	25.043
3	-12.4	3.396	8.186
4	-12.2	2.566	4.430
5	-12.0	19.580	23.312
6	-11.9	2.415	4.072
7	-11.8	20.066	23.020
8	-11.8	23.613	26.540
9	-11.7	23.574	26.636

The result of docking with the ligand molecule hemin

Table 4

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-9.4	0.000	0.000
2	-9.4	3.161	5.170
3	-9.3	18.643	21.529
4	-9.3	3.431	6.919
5	-9.2	19.052	21.927
6	-9.2	3.091	6.846
7	-9.2	3.185	5.405
8	-9.2	0.530	4.269
9	-9.1	2.547	4.597

The result of docking with the ligand molecule protoporphyrin IX

Table 5

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-9.2	0.000	0.000
2	-9.1	3.176	5.255
3	-8.9	3.436	7.066
4	-8.9	18.434	21.465
5	-8.9	0.623	4.367
6	-8.9	18.848	21.916
7	-8.9	3.115	6.996
8	-8.9	3.183	5.477
9	-8.8	2.078	4.815

The result of docking with the ligand molecule pyrimethamine.

Table 6

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-8.6	0.000	0.000
2	-8.2	2.397	2.924
3	-7.9	2.763	4.381
4	-7.6	11.620	12.545
5	-7.6	14.687	15.384
6	-7.5	12.651	13.507
7	-7.5	15.802	16.619
8	-7.4	11.888	12.796
9	-7.3	18.052	19.187

The result of docking with the ligand molecule quinidine.

Table 7

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-4.1	0.000	0.000
2	-4.0	19.885	20.080
3	-3.7	23.606	23.963
4	-3.5	17.035	17.234
5	-3.5	13.298	13.755
6	-3.5	11.208	11.453
7	-3.4	14.870	15.049
8	-3.4	17.557	17.829
9	-3.4	23.387	23.806

The result of docking with the ligand molecule quinine.

Table 8

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-9.3	0.000	0.000
2	-8.8	1.101	2.188
3	-8.8	1.389	2.334
4	-8.7	2.031	5.651
5	-8.1	9.891	12.562
6	-8.1	1.854	5.789
7	-8.0	9.754	12.580
8	-8.0	10.382	12.068
9	-7.8	9.654	11.720

The result of docking with the ligand molecule S hexylglutathione.

Table 9

Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-8.6	0.000	0.000
2	-8.4	13.009	14.418
3	-8.2	2.244	6.222
4	-7.8	2.837	6.390
5	-7.7	11.875	13.908
6	-7.3	12.129	13.686
7	-7.1	11.778	13.184
8	-7.0	13.137	13.831
9	-6.9	13.018	14.754

The result of docking with the ligand molecule tetracycline.

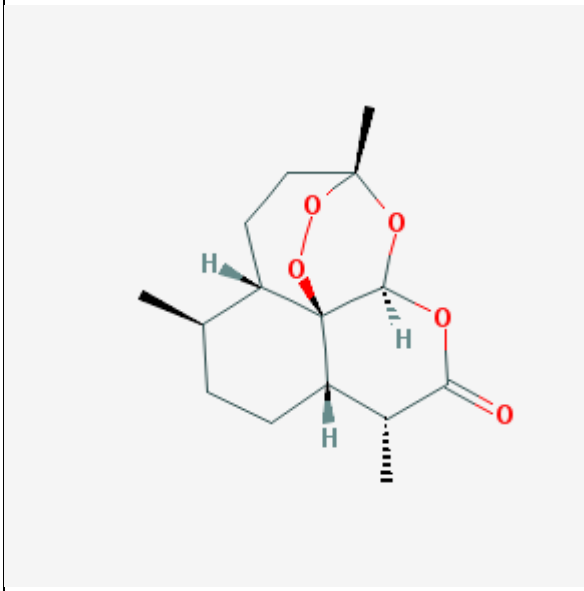
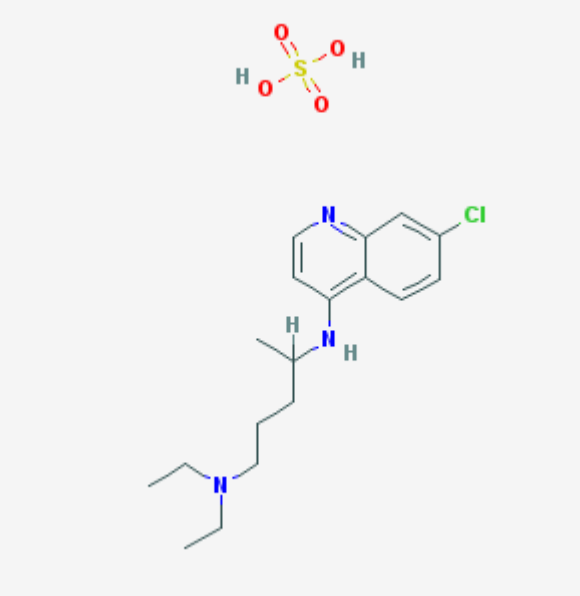
Table 10

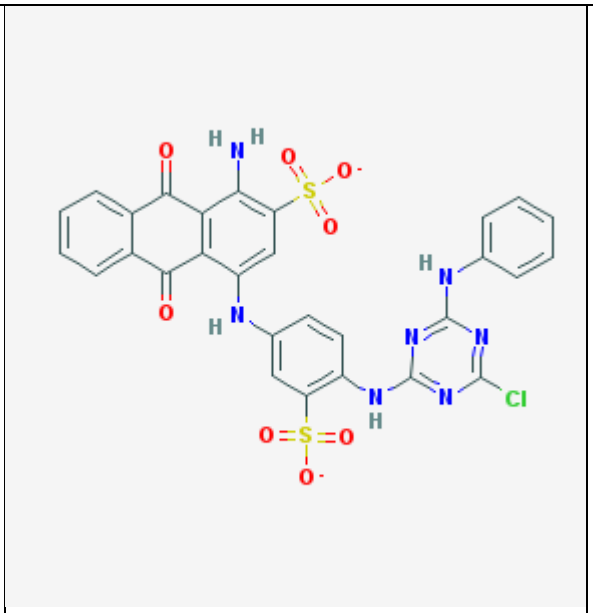
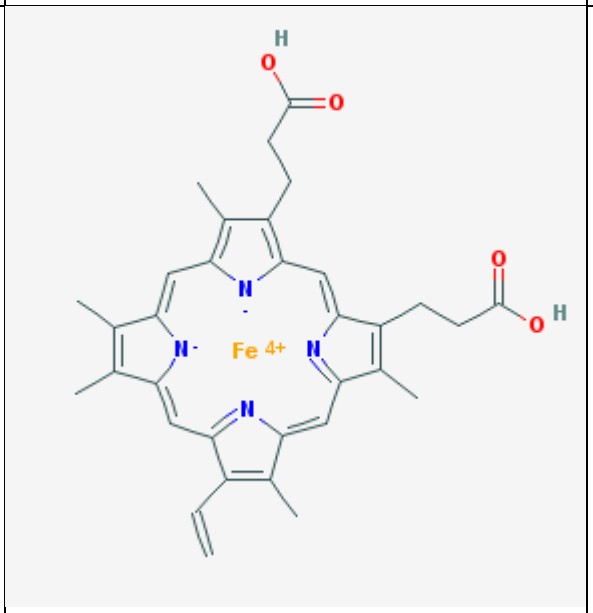
Mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-11.1	0.000	0.000
2	-11.0	19.342	20.173
3	-10.8	19.280	20.016
4	-10.5	18.911	19.552
5	-10.4	20.721	21.150
6	-10.2	19.445	21.184
7	-10.2	19.605	20.672
8	-10.0	2.186	5.974
9	-10.0	18.979	19.765

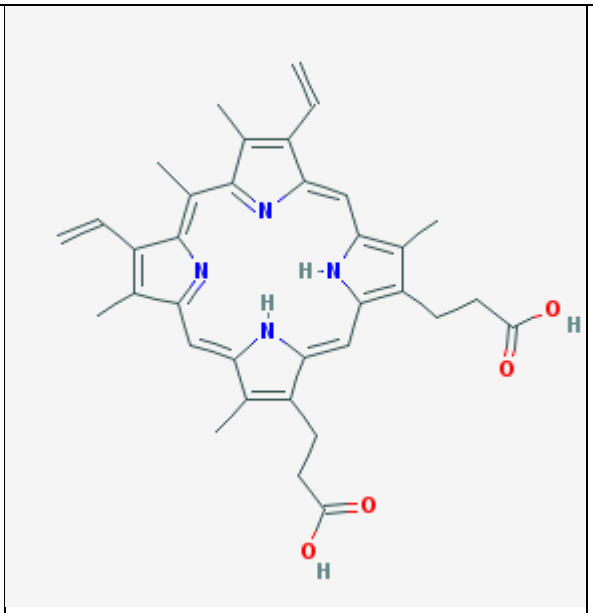
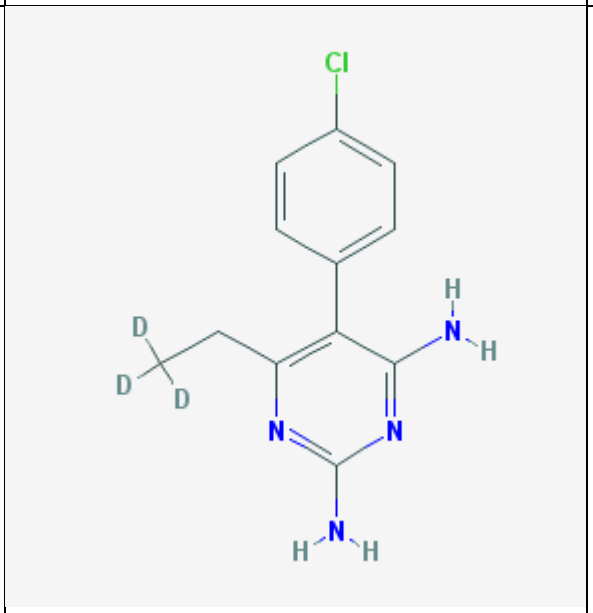
The docking is done with various small ligand molecules which are similar in structure with the already available drugs and the results were noted

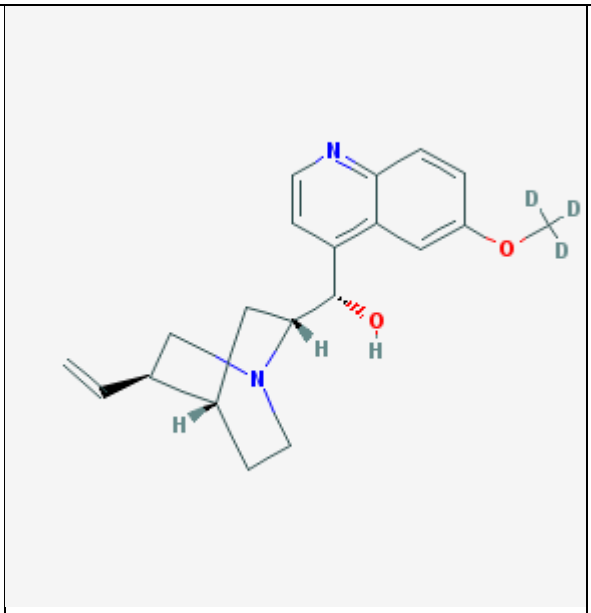
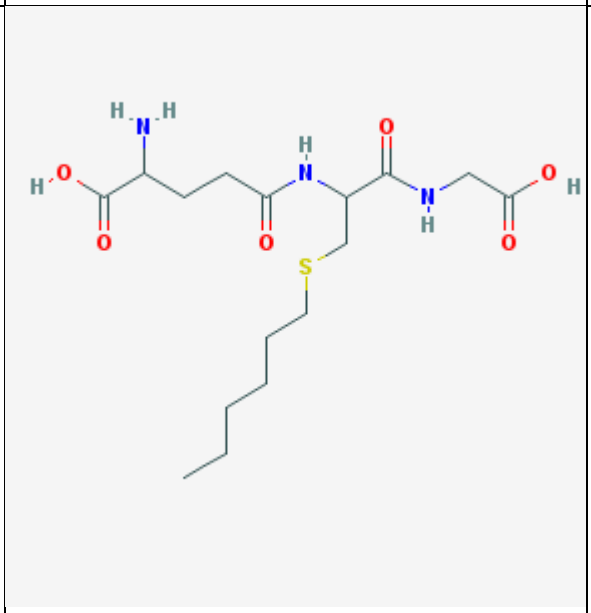
Table 11

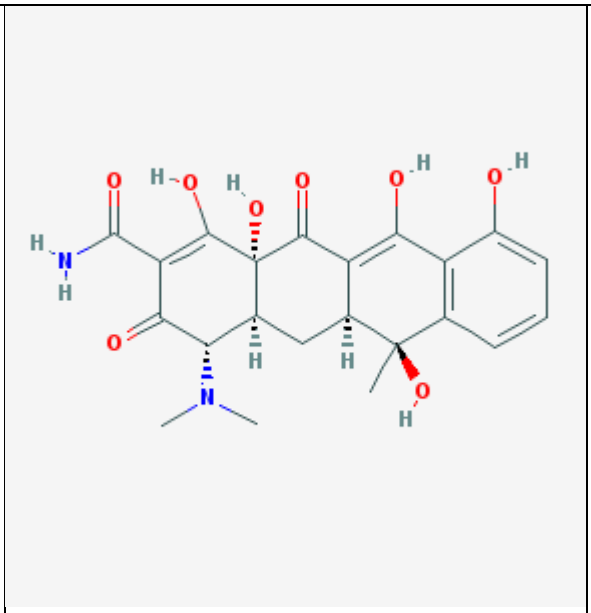
Figure 12. Structures of the small ligand molecules used for Docking.

Serial num.	PubChem ID	Structure of the ligand molecule	Affinity (Kcal/mol)
1	Artemesinine		-11.3
2	Chloroquine		-5.2

3	AGN-PC-0CQS90		-16.4
4	AGN-PC-001O39		-9.9

5	SureCN3819053		-9.4
6	Pyrimethamine-d3		-8.3

7	Quinidine-d3		-9.6
8	Hexylglutathione		-8.7

9	Tetracycline		-17.4

TOXICITY PREDICTION

It is needed for early detection

a) Increasing the cost for drug discovery

b) The need for focussing resources on drug leads and candidates likely to be safe to patient

Toxicity is a major contributor to high attrition rates of new chemical entities in drug discoveries. In this study, an order classifier was built to predict the series of toxic effects based on data concerning chemical-chemical interactions.

Table 12

Serial number	Pubchem ID	Toxicity
1	CID_68827	Non toxic
	CID_71676106	Non toxic
	CID_73265315	Non toxic
2	CID_64927	Non toxic
	CID_83818	Non toxic
	CID_91441	Non toxic
	CID_149423	Non toxic
3	CID_44285647	Toxic
	CID_44456324	Toxic

	CID_59102333	Toxic
4	CID_50921549	Non toxic
	CID_73152295	Non toxic
	CID_73152296	Non toxic
5	CID_59475470	Non toxic
	CID_68801257	Non toxic
	CID_71695767	Non toxic
6	CID_134531	Non toxic
	CID_23423607	Non toxic
	CID_45040307	Non toxic
7	CID_59091406	Non toxic
	CID_68007244	Non toxic
	CID_71751934	Non toxic
8	CID_5141	Non toxic
	CID_14389509	Non toxic
	CID_200556241	Non toxic
9	CID_160964102	Toxic
	CID_162173265	Toxic
	CID_165280009	Toxic

4.2 RESULT DISCUSSION:

The docking of the ligands was carefully observed and their interactions and orientations were also monitored. The results show that **NCGC001161634-07** having a highest binding affinity of -11.3 Kcal/mol, second is the **AGN-PC-001039** with binding affinity of -9.9 Kcal/mol and then **Quinidine-d3** with binding affinity of -9.6 Kcal/mol. All the ligand molecules with high binding affinity than the ATP molecule, which was kept as control to row down the molecules. Hence these molecules which are nontoxic and having a high binding affinity can be used as glutathione s transferase inhibitors.

The ligand molecules which are having a higher binding affinity than the molecules of artemesinin and quinidine cannot be used as the potential inhibitors of glutathione s transferase as they are found to be toxic in nature.

The result of the docked molecules are shown below

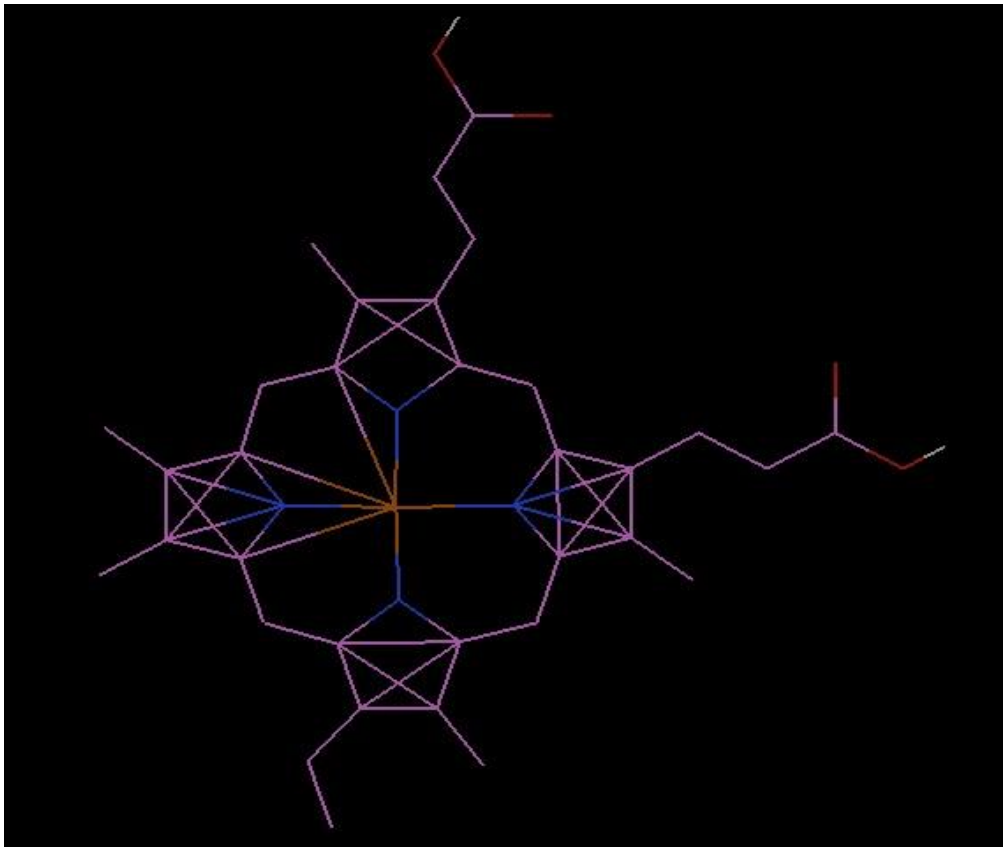


Figure 13. AGN-PC-001039 docking result in Python Molecule Viewer.

Figure 14. Quinidine-d3 docking result in python molecule viewer.

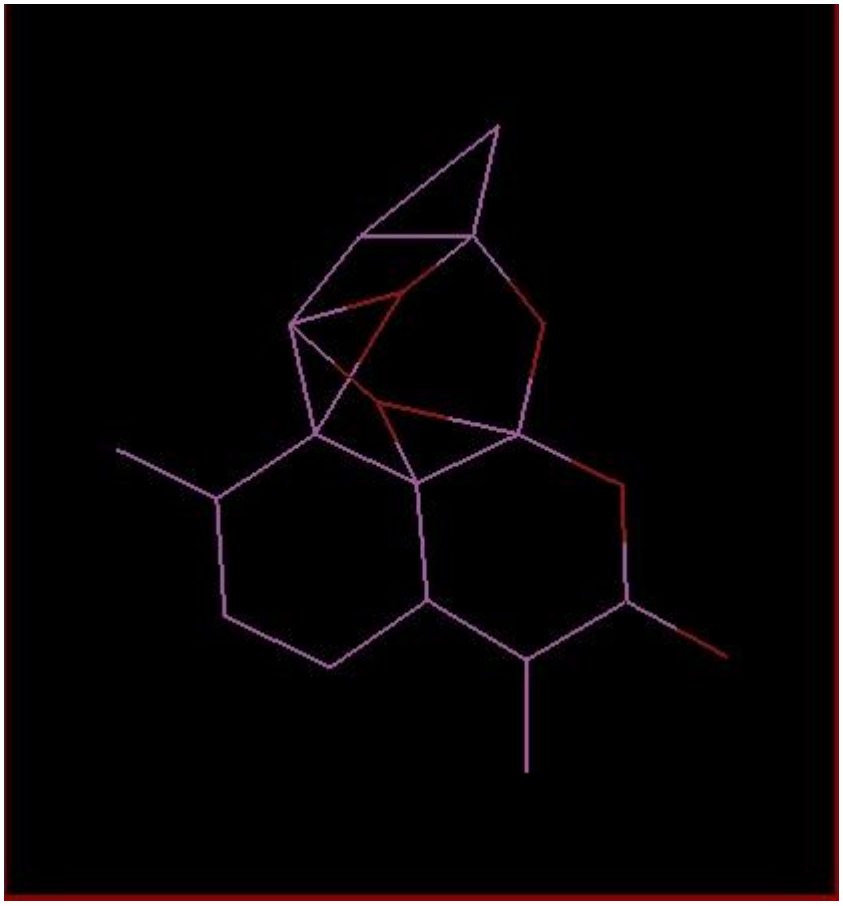


Figure 15. NCGC001161634-07 result in Python Molecule Viewer.

4.3 CONCLUSION:

In the current study we found out that the small ligand molecules that are used for the process of docking are bound to the Glutathione S Transferase in the vicinity of the Tyrosine region. Glutathione S Transferase is a promising drug target for the inhibition of the malarial disease. The small ligand molecules like S Hexylglutathione, Quinine, Pyrimethamine, Tetracycline, Protoporphyrin IX and Cibacron Blue revealed strong binding but with less docking score. The *in silico* docking study validates inhibitory activity of Glutathione S Transferase with molecules like **AGN-PC-001O39**, **Quinidine-d3** and **NCGC001161634-07.** Hence these compounds are novel and alternate drug therapy for the treatment of malaria through the inhibition of Glutathione S Transferase. Further studies are required to mark them as compounds for the development of drugs against malaria.

REFERENCES

1. Nam, Tae-gyu, et al. "A chemical genomic analysis of decoquinate, a Plasmodium falciparum cytochrome b inhibitor." *ACS chemical biology* 6.11 (2011): 1214-1222.
2. Bjelic, Sinisa, et al. "Computational inhibitor design against malaria plasmepsins." *Cellular and Molecular Life Sciences* 64.17 (2007): 2285-2305.
3. Daily, J. áP, et al. "Distinct physiological states of Plasmodium falciparum in malaria-infected patients." *Nature* 450.7172 (2007): 1091-1095.
4. Kalani, Komal, et al. "In Silico and In Vivo Anti-Malarial Studies of 18β Glycyrrhetinic Acid from Glycyrrhiza glabra." *PloS one* 8.9 (2013): e74761.
5. Cowman, Alan F., and Brendan S. Crabb. "Invasion of red blood cells by malaria parasites." *Cell* 124.4 (2006): 755-766.
6. Marrero-Ponce, Yovani, et al. "Ligand-based virtual screening and in silico design of new antimalarial compounds using nonstochastic and stochastic total and atom-type quadratic maps." *Journal of chemical information and modeling*45.4 (2005): 1082-1100.
7. Guantai, Eric, and Kelly Chibale. "How can natural products serve as a viable source of lead compounds for the development of new/novel anti-malarials."*Malaria journal* 10.Suppl 1 (2011): S2.
8. Batista, Ronan, Ademir De Jesus Silva Júnior, and Alaíde Braga De Oliveira. "Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products." *Molecules* 14.8 (2009): 3037-3072.
9. Harwaldt, Petra, Stefan Rahlfs, and Katja Becker. "Glutathione S-transferase of the malarial parasite Plasmodium falciparum: characterization of a potential drug target." *Biological chemistry* 383.5 (2002): 821-830.
10. Fritz-Wolf, Karin, et al. "X-ray structure of glutathione S-transferase from the malarial parasite Plasmodium falciparum." *Proceedings of the National Academy of Sciences* 100.24 (2003): 13821-13826.

: